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(54) Title: PROCESS FOR PRODUCING BIOCHEMICALS

(57) Abstract

This process utilizes a number of small volume compartments containing culture medium (22). Each compartment (22) be enclosed in a tube, preferably transparent, and is segregated in the tube with the aid of a gas-permeable carrier fluid (60). Movement of fluid in a given compartment along the tube to a detection station (40) is often practical. If the fluid in a given compartment is found to contain components desirable for further processing, it can be segregated for such processing. The process is particularly valuable in production of monoclonal antibodies and in use wherein gas-transfer is required to or from the reaction compartment. However, the invention can also facilitate intracellular studies, e.g. pH and calcium content, and studies of the properties of sites on the cell membrane.

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PROCESS FOR PRODUCING BIOCHEMICALS

RELATED INVENTION

This Application is a continuation-in part of the commonly-owned and copending Application filed on 9 October 1987; Serial No. 107,251; by Dennis D. Pietronigro, John L. Sternick, Robert J.Ruemer and Mary Lou Mattes and entitled PROCESS FOR DETECTING BIOCHEMICAL SPECIES AND APPARATUS.

BACKROUND OF THE INVENTION

This invention relates to a novel process for making chemicals, especially biochemicals, and particularly cells in a viable state, in very-small-volume reaction compartments. The invention is also useful in rapid and efficient comparison of large numbers of samples of a given chemical with respect to their differing reaction to a large number of chemicals or concentation of a chemical. A further use for the invention is to facilitate study of cell properties whether those associated with the cell membrane or cell activities carried out within the cell membrane.

There are a large number of processes for making chemicals, including biochemicals, in a number of reactors. However, few of the reactors facilitate detection of small quantities of product while it is in the reactor and most of the prior reactors are relatively comlplex. Others do not differ from conventional chemical reactors. Some processes utilize means to encapsulate reactants.

One kind of serial compartment-processing apparatus, (but not believed to have been utilized for facilitating processing of living, growing and dividing cells or maintaining non-growing cells in good mechanical condition for evaluation procedures, or merely processing chemical samples which utilize gas transfer) is described in U.S. Patent 3,479,141 to Smythe et al wherein chemical samples to be annalyzed were transported, free of intolerable crosscontamination, along a conduit. That patent contained no suggestion for use of the apparatus

described therein as a chemical reactor or as a gas permeable reactor means. Subsequently, a publication ("Capsule Chemistry Technology for High Speed Chemical Analysis" by Cassaday et al in Clinical Chemistry, Volume 31, No.9, 1985; Page 1453) appeared which disclosed the use of such apparatus as a reactor by combining adjacent samples of different reactants by using a merger technique. That article was also directed to analytical techniques which are also intolerable of prior contamination from sample-to-sample. A large number of references, cited in the Cassaday et al article, include:

1. Smith J, Svenjak D, Turell J, Vlastelica D. An innovative technology for "random-access" sampling. Clin Chem 28, 1867-1872 (1982).

2. Steindel S, Schoudt P. An assessment of random access fluid technology. J Clin Lab Automation 3, 319-326 (1983).

3. Bromberg IL, Pollard A, Cheng J, Romaschin A. An evaluation of the Technicon RA-1000 random-access analyzer. Clin Chem 30. 281-283 (1984).

4. Schwartz MK, Stateland BE, Coughlin J, et al. Chemical and clinical evaluation of the random access analyzer "RA-1000". Clin Chem 30, 364-368(1984).

5. Snyder LR, Adler HL. Dispersion in segmented flow through glass tubing in continuous flow analysis: The nonideal model. Anal Chem 48, 1022-1027 (1976).

6. Snyder LR. The prediction and control of sample dispersion in continuous-flow analysis. Advances in Automated Analysis, EC Barton et al., Eds, Mediad Inc., Tarrytown,NY 1977. pp 76-81.

7. Chaney AL. Applications of fluid mechanics in continuous-flow systems. In Automation in Clinical Chemistry 1, NB Scova et al, Eds.,Mediad,Inc., White Plains, NY 1968, pp115-117.

All these publications are incorporated by reference in

this disclosure.

Quite independently of the reactor type described above, there is a substantial amount of prior art relating to biochemical reactions for making useful quantities of such products as monoclonal antibodies. See, for example, the book "Monoclonal Antibodies: Principles & Practice" by J.W. Golding, (2nd Edition, Academic Press, Orlando, Florida, 1986).

The above discussion of the backround of the invention was necessarily made with knowledge of the invention. It is not to be inferred from such discussion that Applicants believe any of the art disclosed in the cited references, alone or in combination, would have made it obvious to utilize such art in solving the problems faced by applicant.

As described below, Applicants wish to accelerate the production of such biochemicals utilizing a rapid means for identifying cells useful in making such products.

SUMMARY OF THE INVENTION

It is a general object of the invention to provide a novel process useful in applications wherein it is desirable, for any reason, to carry out gas utilizing biochemical reactions in a series of small compartments which can then be moved, at least relatively fast , under a detection apparatus.

Another general object of the invention is to provide a process for rapidly preparing samples for detection of specific ligand-antiligand reactions especially in the presence of living cells and organisms.

A further object of the invention is to provide means to prepare culture samples for rapid detection of and collection of, secreting cells, e.g. hybridoma cells, and other biological cells and organisms of specific characteristics which are present in said samples.

It is another object of the invention to provide a

novel process as described above wherein functional gas transfer, into or out of the reaction compartments, is possible.

Another object of the invention is to provide means to concentrate growth factors, secreted by cells which are in relatively concentrated forms.

Still another object of the invention is to provide an improved process for manufacturing useful quantities of hybridoma cells and antobodies secreted by such cells.

Another object of the invention is to acheive all of the above objects while maintaining a suitable enviornment for each sample over a prolonged period of time. Another object of the invention is to provide a process that will allow rapid relative concentration of the biological compositions being produced by cells in a culture being processed.

An important object of the invention is to provide apparatus and process for facilitating the study of biochemical entities including, for example, the study of internal cell activity and the study of properties of the cell membrane itself and particular sites thereon.

Other objects of the invention will be obvious to those skilled in the art on reading the disclosure.

The above objects have been substantially achieved by the process described below.

The general type of apparatus useful in performing the process of the present invention is described in the U.S. Patent 3,479,141 to Smythe et al. That patent is incorporated into this disclosure by reference as it gives a general summation of the way in which reactor compartments of chemical composition can be separated and moved along a tube. Smythe et al, cited in said Background section, used their apparatus to transport a series of samples to test apparatus. They suggested nothing about the importance of gas-transfer or the facility with which

transfer may be achieved in such processes as described.

The tubular-conduit aspect of the apparatus used in the process of the present invention resembles the Smythe et al apparatus. However, the following principles, not recognized or utilized by Smythe et al, must be kept in mind for many biological processes.

1) The carrier fluid, which coats the wall of the tubular conduit and also forms walls about the reaction compartments is preferably gas permeable, thereby serving as means to provide gas exchange or transfer into the reaction compartment from an intermediate fluid positioned between sample-bearing reaction compartments, sometimes referred to herein as the "separation fluid" which is often air or air modified by the addition of supplemental gases, e.g. by 5% CO_2.

2) The wall of the tubing is thin enough to transfer process-facilitating gas therethrough, thereby obviating the need for all gas transfer to be along the length of the path within the tube which holds the compartments.

3) The wall can be, and advantageously is, sufficiently translucent to allow optical inspection of the reaction mix.

4) In many biochemical operations; e.g. where a particular secreted product is to be formed, and where the presence of an appropriate secreting cell is questionable, it has been found to be very desirable to provide extremely small reaction compartments which can be screened more quickly and effectively to identify the presence of the desired secretion product and, consequently, the presence of cells suitable for further cultivation within the compartments. It has been found that such reaction compartments can be as small as a fraction of a microliter; although, the invention is operable with much larger samples.

The term "liquid", as used herein, is used to describe any material, however viscous, which can be

moved without substantial damage along the tubular reactor. Thus, many gels are suitable "liquids", suitable for use as cell-bearing culture compositions, as are microcapsules in which the initial seeding cultures have been encapsulated, in the practice of the invention.

In most of the applications of the invention, the apparatus of the invention, and such ancillary apparatus which contacts the fluids being processed, must be sterilized to assure a safe environment for cell-bearing cultures and the like.

Although carrier fluids can be flourochemicals or like materials, which facilitate the movement of compartments can be used. For example, aqueous solutions can be utilized when independent gel-type walls are used in forming compartments. Such gel-type walls can consist of sodium alginate or like materials which would encapsulate the fluid.

Moreover, it should be understood that the present disclosure discusses oxygen-utilizing processes. Nonetheless, it is contemplated that the invention shall also cover anaerobic processes in which case, the separation fluid could be some appropriate non-oxidizing fluid such as nitrogen. Any substantial oxygen transmission into a compartment would be avoided by choice of a suitable gas environment around the processing compartments.

In general, the concept upon which the invention is based is that one can lead to a very quick determination of the presence in a given reaction compartment of suitable secreting cells. This is achieved by using a very large number of extremely small compartments and rapid evaluation, e.g. by relative movement of hundreds of compartments per hour under a detection device. Thus, even though one has reduced, indeed minimized, the probability of suitable antibodies being produced in any given compartment by use of very small, even sub-microliter, compartments, the much more rapid inspection

rate, the lack of a larger mass of reaction liquid components which are not of interest, and the ability of maintain the multitude of samples (usually in excess of 1000, typically in excess of 5000 and often in excess of 20,000) at optimum environmental conditions, combine to form an optimum way to initiate detection and production of desired biochemical products.

Another way of stating this advantage is that, although the secreted product is much less likely to be produced in a given compartment, when it is produced in a compartment it can be detected much earlier because it will be present in a higher, more detectable concentration.

Furthermore, since a large number of compartments are used, the number of cells initially seeded for compartment is very low, Typically less than, or equal to, one secreting cell per compartment instead of the 10 or more cells per compartment usually used in the prior art. The advantage here is that the wanted cells are cloned immediately without the necessity of performing multiple time-consuming, sub-cloning steps.

When reaction compartments are formed in polymer tubing, of the size and type described herein, compartments of a bioculture to be tested can be reduced in volume by using a carrier fluid such as silicon oil of a perfluorocarbon fluid sizes of less than 1 microliter, indeed to about 0.2 microliters or smaller. Carrier fluids are typically about 25 centistokes in viscosity and are obtainable under the trade designation Galden D-25 from the Ausimont Company, a subvsidiary of Montedison, and a number of other suppliers. The reaction fluid is best selected to avoid any substrate sticking of proteins in the compartments to the tube wall. This is especially important in those circumstances wherein it is desirable to move the bubble through the tubing.

The carrier fluid is also found to be useful as means to "block" the surface of the tube which contains reaction

compartments enclosed within perfluorocarbon liquid coating. A one percent (1%) solution of BSA is also useful in such blocking. Other materials can be used but they should not interfere with gas transfer where such transfer is needed. By "blocking" is meant providing a coating which aids in avoiding interaction and excessive sticking of materials within the reaction compartments along the tubes.

The carrier fluid layer between the separation fluid and the reactor compartment can be characterized by the solubility of air therein. Solubilities of 20-50ml of air per 100 ml of carrier fluid is common.

One of the principal advantages of the invention is that the reacting compositions in the compartments can be detected optically, e.g. by measurement of epifluorescence by being measured in situ, through a transparent or translucent wall of the reactor tube, i.e. while the reactants are still within the medium in which the material being detected is being produced.

In a typical situation, the reaction compartments will be constrained within a chemically inert tubing, advantageously a polyfluorocarbon tubing (formed of an FEP (fluorine-ethylene polymer) tubing of 18 gauge, i.e. 0.042 inch inside diameter and .012 inch wall thickness). One such tubing is sold under the tradename Zeus by Zeus Industrial Products, Inc. of Raritan, New Jersey. The tubing illustrated below has an inside diameter of about 0.03-0.04 inches and a wall thickness of about 0.01 to 0.02 inches. Any tubing used should be sterilizable, non-toxic, non-wettable by water, and preferably(for long-term reactions) sufficiently gas-permeable to be useful in transferring at least CO_2 and O_2 between the inside and CO_2 enriched air outside of the tubing. The non-wettability characteristic can be provided by adequate blocking.

It is not necessary to use a monolithic, pore-free tubing material. Porous tubing can be used providing only

that it adequately contains the carrier fluid and that it does not allow contamination of the samples. Whether contamination is a factor depends on the mobility of a specific contaminant through the carrier fluid and, of course, the parameters, particularly the time, during which the compartments are to be processed. In typical situations, the tube material will exhibit permeabilities of 700cc/100in^2-24hr mil thickness for oxygen and 1670cc/100in^2-24hr mil thickness for CO_2. All measurements are at 25^{o}C and astmospheric pressure.

It should be understood that the gas permeability required of the tubing material will vary substantially depending on such factors as the size of the reactor compartment, the rate of biochemical reactions taking place therein, the concentration of reactants etc. In some cases, reactions can be carried out for many days without such gas transfer. However, it has been noted that for many purposes, a tubing material is useful which permits about 500 cc of oxygen per 100 square inch area per mil of tubing thickness per 24 hours and 1500 cc of CO_2 per 100 square inch area per mil of tubing thickness per 24 hours. Such measurements are made under standard conditions of temperature (23^{o}C) and pressure 14.7 psig. The carrier liquid permeability is usually discussed in terms of "air solubility" in the liquid. Fluorinert FC-71 will dissolve about 20 ml of air in 100 ml of liquid (standard condition of temperature and pressure). Materials with such characteristics appear to be entirely suitable for use in the invention. Other such materials will dissolve still more air and are very permeable to gas. In general, "permeabilities" of above 10 ml of air in 100ml of liquid tend to be suitable for most biochemical reactions of interest when applied in thin coatings to block tubing and act as a carrier fluid.

Such permeability will allow sustained cell growth and division, in typical cases, for many days, and even for weeks. One typical example would be the sustenance and division of, say, ten hybridoma cells initially contained

in a 2.5 microliter reactor compartment for about a week at 37°C when the reactor is kept at 37°C in a 5% CO_2/95% air environment for about a week.

It is to be noted that shaping of the compartments so that the compartment is spherical, or better still, somewhat elongated in the preferred direction of a beam of light from detecting apparatus, i.e. the direction perpendicular to the axis of the tubing is advantageous. Such shape and orientation are desirable because it makes the sample more concentrated along the light beam used in for example, epireflective optical detection techniques known to the art. This is of particular advantage with very small compartments, e.g. those below a mocroliter in volume, and particularly below 0.5 microliters, where the material to be detected may first appear in very small quantities.

The merger of reactor compartments in a tube-type reactor, a feature known in the art to bring reactants together to form a single reaction compartment, has a special use in the biochemical processes of the invention. This is so even though each compartment initially contains a reaction mix taken from the same culture medium and ostensibly of the same initial composition. In practice, however, when one uses ultra-small compartments, there is usually a cell of one type within one compartment which does not appear in adjacent compartments. Thus, in a circumstance wherein (1) an antibody of desired specificity appears in one initial compartment (thus indicating the presence of at least one desirable secreting cell, but more probably, the proliferation of such desirable cells); and wherein adjacent compartments do not indicate the presence of the cell; one can merge the compartments to provide additional nutrition for the contents of the reactor compartment having the appropiate secreting cell. Of course, in many applications, for example cloning applications, the adjacent compartment should be free of interfering cellular matter.

In addition, merging compartments can be used to add additional reagents, for example, those that influence cell growth, division of biochemistry of the cell. Some such reagents are growth inhibitors, growth factors, and drugs or chemicals specific for cell-surface receptors. Also, an additional cell-type could be added to a culture containing a first cell type to study the cytotoxicity or other effect, e.g. effect on growth and differention of one of the cell types by the presence of a second cell type.

When the process is carried out in a attempt to grow polyclonal-(or monoclonal-)secreting hybridoma cell, in glass tubing, the yields of cells were very low. This condition was improved somewhat by using a conditioned media. However, when using the permeable gas tubing and carrier fluid, at least a magnitude greater cell growth was achieved even with non-conditioned media.

It should be understood that the gas-permeability feature of the apparatus can be dispensed with for short-term use of the process described herein. Indeed, even glass can be used as the tubing, in some situations which either do not require supplemental gas or can rely on gas transfer along the axial direction of the reactor, but not without suffering from the problems discussed above.

By "conditioned media" is meant a culture media which has been used in a preliminary process to grow cells, typically hybridoma or myeloma cells. The media is then centrifuged to remove solid matter from the media. Then it is used as a "conditioned" culture media. As a result of its conditioning during the preliminary process, the cell contains a, or some, additional growth-promoting factors, e.g. such as B-cell growth factors. Clearly use of conditioned media is useful, but it is a special advantage of the process that non-conditioned media may be utilized with very good results.

Typical process times for culture media which are being used to produce polyclonal or monoclonal antibodies

are 10-30 days. Depending on the detection set up, and the propensity of a particular culture medium to result in sticking of compartments, it is preferred to move the compartments through the tube except for relatively short periods of time, say up to about 20 hours or so. In the preferred practice, of course, it is desirable to keep the compartment stationary for a substantial period of time, say several hours or more, even if only to shorten the length of tube required.

Proteinaceous materials in the compartments tend to stick to the tube, if static for a period of time, notwithstanding the presence of carrier fluid. There are a number of solutions to alleivate this inconvenience. One is to keep the cells moving except for short stops as described below. Another is to select a relatively light carrier fluid, e.g. a carrier fluid filled with microballoons, e.g. glass microballoons, preferably of sub-micron size. These can form means to reduce the effective density of the carrier fluid. Such microballoons can also be used as glass beads to form a floating patch when included in the media compartment.

The selection of carrier fluid can be somewhat broadened by mounting the tube reactor on a surface which is rotated at a rate which can be as slow as one revolution per minute or even much slower, so long as gravity does not allow the separation media to float or sink relative to the reaction compartments.

The process of the invention is particularly advantageous in allowing the processing of living and dividing cells in vitro. However, it is also advantageous in processing cells like white cells, primary liver cells and the like which do not reproduce <u>in vitro</u>, because it is so mechanically gentle that the cells are preserved during processing before detection. For example, one may wish to study the effect of some characteristics of such cells as a function of temperature, time, gas atomsphere, etc. In such a case, the apparatus of the

invention is valuable, especially in processing large number of cell samples, each in a different compartment.

Also, the invention can be used in any number of reactions wherein it is desirable to test the response of compartmentalized reactants to a gas environment. Thus, for example, one can study the effect of oxygen on a free-radical, e.g. the electron transfer of adriamycin (doxorubicin) radicals to tetrazolium salts. Also, one might want to study the electrontransport in mytochondria with respect to the gas media or thermal environment. In such cases, samples for analysis could be processed while being transported through the tubing and under a detector. The gas environment within which the reaction takes place could be varied, from time to time. Study of the phenomena could be made by any appropriate detection or analysis.

The tubing, of course, is usually enclosed within a housing containing an environment suitable for the investigation, thereby providing a way to achieve the proper thermal or chemical environment within the tube.

There are many processes wherein movement through a tube is not necessary after the tube has been loaded with the compartments. Thus, for example, one may wish to add different growth factors to different cell compartments (selected to have the same kinds of cells therein) for the purpose of evaluating the effect on the behavior of the cells. This is merely one type of analytical process wherein the tubular conduit, with the compartments still in it, can be discarded because, very often, once the analysis is made, there is no desire to keep the analyzed material for further use. Indeed, a major advantage of the invention is to keep any cell population in a compatible environment during the period it is being subjected to a particular process.

Among other types of cell-studying processes are cytoxicity assays wherein drugs (or toxins) of different kinds and/of different quantities can be put into

different compartments within a tube and, after an appropriate time, the different cells can be investigated for the effect on the cells. Human leukocyte antigen (HLA)- typing is also facilitated by the use of the invention. Various antisera active with different HLA subtypes are placed in different compartments (containing the same cells). Killing of a cell within a compartment is indicative that the cell-line being evaluated is vulnerable to the particular antiserium which was contained in its compartment. Death of a cell can be determined by fluorescent means or other detection means well known in the art.

One way of determining the death of the cell, say when it is evaluated with respect to the presence of a specific drug, is to utilize a material such as carboxyfluorescene diacetate (CFD). Only living cells will cleave off the diacetate and become flourescent. Thus, a tumor line can be labelled with CFD, then worked, and seeded into cultures with drugs of interest. All cells will light up, but killed cells will allow the fluorescent tag associated therewith to leak out of the cell, become diluted in the media, and lose its fluorescent character. Thus the living cells can be detected by fluorescent means.

Intracellular studies can be readily facilitated by the use of the invention. For example, one may wish to measure the pH, or free calcium, or a particular enzyme within a cell. pH measurements can be done by using appropriate pH sensitive dyes, e.g. a fluorescent material, BCECF (supplied by Calbio Chem. of San Diego), within the cell, and after a proper amount of time in which the cell is subject to the physical or chemical conditions to be evaluated, the quantity or color of the dyes can be optically evaluated as an indication of the pH. Somewhat similarly, the intracellular environment can be explored for free calcium by tagging it with FURA-2, (supplied by Molecular Probes Company of Junction City, Oregeon).

Cell surface markers (tags) such as supplied by the Ortho Diagnostics Company, are also useful in studying cell-membrane parameters.

It is pointed out that the above-indicated analytical techniques are not, standing alone, part of the invention, because they are known in the prior art, but merely illustrative of the kinds of processes in which the apparatus and process of the invention find advantageous utility even when actual recovery or harvesting of a cellular product is not required.

Intracellular enzyme status can be measured, e.g. acidbeta-galactosidase, is determined by using the fluorogenic substrate fluorecein di-beta-d-galactopyranoside (Cytometry Vol. 7 Pages 483-486, 1986).

Of course, it is also possible to carry out various other time-dependent and non-predictable processes than purely biochemical processes according to the invention. For example, one can carefully monitor the growth of slow-forming inorganic or organic crystals being produced, of the production of other chemicals.Each compartment can contain a somewhat different chemical composition so that, for example, the effects of various catalyst levels can be noted. The transparent wall allows most optical measurements to be made in situ and this is a special avantage of the process particularly when the reaction compartments are very small. The sticking or compartment "hopping" phenomena are not usually present in such applications because of the lack of proteinaceous material. The gas permeability of the apparatus also facilitates choice of environment for any reactions or a study of the reaction under a series of gaseous environments.

ILLUSTRATIVE EMBODIMENT OF THE INVENTION

For simplicity, the process of the invention is described with reference to the production of an antibody

ligand as an indication of the presence of a desired secreting cell being produced in a reaction compartment. This involves the use of a single antiligand, i.e. one specifically useful in detection of the kind of antibody ligand to be produced.

In this application there is described a preferred embodiment of the invention and suggested various alternatives and modifications thereof, but it is to be understood that these are not intended to be exhaustive and that other changes and modifications can be made within the scope of the invention. The suggestions herein are selected and included for the purposes of illustration in order that others skilled in the art will more fully understand the invention and the principles thereof and will be able to modify it and embody it in a variety of forms, each as may be best suited to the condition of a particular case.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic diagram showing a liquid biological culture containing hybridoma cells composition isolated in a compartment within a plastic tubing, best seen in Figure 7.

Figure 2 illustrates the same culture as shown in Figure 1 after antibodies are expressed by said cells.

Figure 3 is indicative of the same culture after there is substantial binding of both antibodies and a fluorescent probe to magnetic beads.

Figures 4, 5 and 6 show the magnetic material pulled into an opaque patch at the side of the compartment.

Figure 7 illustrates, schematically, the typical tube processing-apparatus for handling a series of biochemical compositions.

Figure 8 is a schematic diagram indicating how a tube reactor is rotated.

Figure 9 is a schematic illustration of favored

shapes of compartments in which the process of the invention is most favorably carried out when it is desired to optically inspect the reaction compartments during processing.

The following example, in conjunction with Figures 1 through 6, illustrates an in situ homogeneous assay for detecting the presence of an antibody secreted by hybridoma cells in a tissue-culture media. The tubular arrangement of reactor compartment is similar to that employed by Smythe et al in U.S. Patent 3,491,141 and in Figure 7 of this Application. Appropriate sterilization of the tube and perfluorocarbon carrier liquid should be accomplished before use.

EXAMPLE 1

This assay demonstrates the detection of an antibody having size in excess of 100,000 Daltons.

As seen in Figure 1, polystyrene magnetic-beads 20, of nominal 1.75 micrometer diameter and supplied by the Seragen Company are coated by absorption with goat anti-mouse (GAM) IgG antibody (heavy and light chain type obtained from the Zymed Company) are used. Bovine serum albumin (BSA) was used to block any further reactive sites on the magnetic-beads. Beads 20 will serve as a substrate for a probe which will be bound to the beads and eventually pulled into an opaque patch. They were dispersed in a series of tubing-constrained reactor compartments such as 22, which contains a tissue culture medium 24. Also dispersed in compartment 22 is a so-called tag chemical, or probe, 26 which is an FITC-tagged goat anti-mouse polyclonal antibody, also obtained from Zymed, and some preselected hybridoma cells 28 (55.2 hybridoma cells producing IgG_{2a}). Before use, the cells are washed about four (4) times with Hanks balanced-salts solution to remove any free antibody. Other reactor compartments are prepared to contain either nonsecreting 653 mouse myeloma cells as negative control or IgG_{2a} mouse myeloma protein (a product of ICN Corp.) which is used as a positive antibody

control. Note the tubing 52 is held within an outer housing 21 which contains a gas atmosphere suitable for maintaining the desired atmosphere over the tube.

Figure 2 indicates a new situation wherein, after the passage of time, and antibody 30 is secreted from at least some of the cells 28, i.e. from a secretor cell.

Magnetic-beads 20 through their coatings of goat anti-mouse antibody, which may be called MB/GAM, bind to the newly-secreted antibody 30 (which may be called Ab) as does the "probe" FITC-tagged goat anti-mouse polyclonal antibody 26 (which may be called GAM). The resulting complex is shown in Figure 3 as a chemical composition 32 of the formula using the terms set forth above:

MB/GAM- Ab- FITC/GAM

The probe-antibody 26 only associates with beads because of the mouse antibody thereon. In Figure 3, "p" is the combined "composition" of magnetic bead, secreted anitbody, and the probe.

As seen in Figures 4 through 6, a magnet 40, a 7 kilo-gauss magnet which is about 1x1.4x7cm in size, is used to pull the magnetic-particle bearing material over to a small area of the perimeter of compartment 22 and thereby to provide a substantially opaque population of such beads in a patch 42 as seen in Figure 6.

The quantity of fluorescence emitted in response to a suitably-matched, stimulating light, as known in the art, will necessarily be directly related to the quantity of antibody secreted by cells 28 and which attach to both the MB/GAM and the FITC/GAM to form the MB/GAM - Ab- FITC/GAM material. Of course, if there is any magnetic material which has yet to attach to an antibody, it too will be contained in the magnetic patch 42, but it will not contribute to fluorescence.

Figure 7 is schematic view of a typical, sequential, processing procedure utilizing, in general, a known scheme wherein compartments 22 of about 0.3 microliter in volume

to be subject to analysis are moved through a polytetrafluoroethylene tubing 52 separted by gas bubbles 50. A liquid material, a perfluorocarbon liquid composition, and sold under the trade designation Fluorinert FC-77 by the 3M Company, provides means to facilitate separation and also to facilitate a lubricated, progressive movement of the compartments along the interior wall of the reaction tube 52. Tubing 52 forms means to allow sufficient nutrient gases and CO_2 to permeate therethrough. The gas or air bubbles between the compartments can form additional surface for transportation of gases through the Fluorinert carrier phase 60 which surrounds both the gas bubbles and the compartment containing the biological culture. The clinical procedures are as follows:

All compartments contained treated magnetic-beads in a typical concentration of about 1.12×10^8 beads per milliliter. The concentration of beads can be much lower. The probe, is added at a concentration of 375 nanograms of FITC/GAM per ml. From 4 to 250 hybridoma or non -secreting myeloma cells were initially placed in appropriate reaction compartments. The IgG_{2a} mouse myeloma protein, supplied by ICN Corp., also combines with the magnetic-beads and fluorescent probe and is used as a positive control. It was placed in the other reactors at a concentration of 37.5 nanogram per mil.

The interior of the tubing was lightly coated with a carrier fluid, i.e. that sold under the trademark Fluorinert FC-77, a composition that is sterilizable, wets the tubing, is gas permeable and must have suitable transparency to enable the optical assay. The reaction mixture was sucked from different wells into the Teflin tube using a manifold and a syringe pumping system, thereby forming reactor compartments within the tube. Sufficient FC-77 was also pumped into the tube so it formed a barrier layer about the compartment of the bioculture, i.e. the reaction mixture. Air was introduced to the tubing, as known in the art, to separate

adjacent compartments. The compartments within the tube were then placed in an incubator at 37°C and in a 5% CO_2-95% air environment for 24 hours. Thereupon magnetic-beads were pulled into an opaque patch and the fluorescence of the patch was determened as a means of the presence of the desired ligand.

It should be understood, depending on the pricrities of the processors, a detection of a useful biochemical indication of the existence of a suitable secreting cell in a compartment can be immediately followed by a recovery of the desired compartment by sucking the compartment to the exit thereof, and isolationg the cell therefrom, e.g. by cutting out the compartment from the tube, or sucking it out with a microsyringe, or in an appropriate situation, moving it through the tube to an exit port. Alternately, it may be desirable to merge adjacent compartments into the desired compartment by procedures known in the art. This alternative forms means to bring additional nutrients to the useful chemicals in the desired compartment. It is to be noted that, upon such merger, it is often convenient to transfer the merged material into a larger diameter tube, thereby maintaining a preferred gas-transfer area relative to the volume of the larger compartment resulting from the merger.

All reactors from wells containing either secreting cells or IGg_{2a} were positive for fluorescence. Compartments with nonsecreting cells or media only were negative for fluorescence.

Figure 8 shows a reactor tubing 52 being rotated about an axis A-A to avoid excessive flotation or settling of the solid components in the reaction compartments. Usually such rotation is not required except when the reaction compartments are to be at rest for several hours rather than being moved slowly through a tubing.

Figure 9 is a schematic view showing nearly-spherical compartments 91 separated by air bubbles 92 in a plastic

tubing 93 with the perfluorocarbon liquid 94 facilitating the separation of the compartments and bubbles, and facilitating movement of the bubbles, and especially compartments, along the tube 91.

The patch-drawing aspect of the invention has been discovered, by one of our co-workers, to be of particular value and utility in that the agglomeration of the patch even without a tag (e.g. of the general type drawn into a fluorescing patch in the preceding example) has been found to be detectable to the eye, usually with the help of a microscope at, e.g. 100-200X magnification and without the aid of labels. This phenomena, best carried out with magnetic particles, is based on a difference of texture between the two samples. The material which has picked up the antibody (e.g. the MB/GAM - Ab - FITC/GAM of the preceding example) has a more dense ,more compact appearance than do the beads when they have yet to be complexed with a material of interest. An example is the MB/GAM state in the foregoing example. This procedure, even if less sensitive than the tagging procedure, may ultimately prove to be a preferred way to simplify the process of the invention.

It is also to be understood that the following claims are intended to cover all of the generic and specific features of the invention herein described and all statements of the scope of the invention which might be said to fall therebetween.

Having described our invention, what we now claim is:

CLAIMS:

1. A process for promoting growth and production of biochemical products in cell culture media which includes:

(a) placing a series of cell culture media samples in the form of spaced reaction compartments along a tube;

(b) treating the wall of said tube with a gas-permeable carrier liquid;

(c) maintaining separation of said reaction compartments within said tube with an intermediate separation fluid;

(d) forming a gas-permeable barrier of a carrier liquid between said cell culture medium compartments and said separation fluid;

(e) incubating said series of cell culture media and inspecting said media to detect the presence of desired biochemical products of the incubated compartments; and

(f) recovering said desired biochemical products from at least some of said spaced reaction compartments.

2. A process as defined in Claim 1 wherein said compartments are moved along said tube during said incubation step.

3. A process as defined in Claim 2 wherein said culture media and said recovered biochemical products each comprise living cells.

4. A process as defined in Claim 1 comprising the step of placing a cell culture media of less than about 10 microliters in volume into said reaction compartments.

5. A process as defined in Claim 2 wherein size of said reactor compartments, the size and shape of said compartments, and the thickness of said carrier fluid is selected to provide sufficient O_2 and CO_2 gas exchange between said separation fluid and said reactor compartment to enhance the growth ratio of cells.

6. A process as defined in Claim 4 wherein said tube wall is sufficiently thin to allow gas to enter said tube through the walls thereof and to permeate the carrier

fluid in quantities effective to promote cell growth.

7. A process as defined in Claim 1 wherein said liquid composition comprised at least one secreting cell and wherein said recovery step includes the recovery of said secreting cells.

8. A process as defined in Claim 1, 2, 3, 4, 5, 6, or 7 wherein said process is operated with a cell culture comprising a non-conditioned media.

9. A process as defined in Claim 1, 2, 3, 4, 5, 6, or 7 wherein said reaction compartments are less than 1 microliter in volume.

10. A process as defined in Claim 1 wherein said tube is transparent and comprising the additional step of optically evaluating a said compartment through said wall of said tube as said tube is moved relative to an optical evaluation apparatus.

11. A process as defined in Claim 1, 2, 3, 7, or 10 wherein said reaction compartments are not elongated along the axis of the tube.

12. A process as defined in Claims 1 or 7 comprising the step of detecting an antibody indicative of a suitable hybridoma cell in a first reactor compartment, and merging the contents of the first compartment with at least one other compartment which is free of cellular material to promote an improved rate of formation of additional said suitable hybridoma cells and secretion of said antibodies by said cells.

13. A process as defined in Claim 9 wherein said compartments are of a maximum volume of 0.5 microliters.

14. A process as defined in Claims 1, 2, 3, 5, 7, 10, or 13 wherein a large number of said compartments each comprise liquid taken from a common source composition.

15. A process for promoting the growth of biochemical products in cell culture media comprising the steps of:

(a) placing a series of cell culture media in the form of spaced reaction compartments along a tube;

(b) treating the wall of said tube with a carrier liquid which is means to facilitate movement of said compartments along said tube;

(c) separating said reaction compartments within said tube with an intermediate separation fluid;

(d) incubating said media in said tubes;and

(e) recoving said biochemical products of at least some of said spaced reaction compartments at the end of said conduit.

16. A process for evaluating the effect of a gaseous and thermal environment on a chemical reaction comprising the steps of:

(a) placing a series of reaction media, containing reactants for said chemical reaction, in a series of spaced reaction compartments in a gas-permeable tube;

(b) maintaining said tube, sequentially in a plurality of different environments, predetermined as to temperature and gaseous composition;

(c) treating the wall of said tube with a gas permeable carrier fluid which is means to facilitate movement of said compartment along said tube;and

(d) observing the effect of said environment on said chemical reaction.

17. Apparatus for incubating cultures, said apparatus comprising:

(1) a housing within which is maintained a cell-growth-promoting invironmint of gases;

(2) a tubing in said housing, walls of said tubing being gas-permeable permitting gases to permeate therethrough into said tubing; and

(3) a gas-permeable carrier fluid along the walls of said tubing, also forming means to permit gases to permeatae therethrough.

18. Apparatus as defined in Claim 17 further comprising within said tubing a series of sample-enclosing walls formed of a gas-permeable carrier fluid.

19. A process for promoting and analyzing change in cell culture media comprising the steps of

(a) placing said media in a series of spaced reaction compartments along and within a tube;

(b) treating the wall of said tube with a gas-permeable carrier liquid;

(c) maintaining separation of said reaction compartments within said tube with an intermediate separation fluid;

(d) forming a gas-permeable barrier, of a carrier liquid, between said compartment and said separation fluid;

(e) incubating said series of cell culture media; and

(f) inspecting said media to determine the condition of biochemical components within said cell.

20. A process as described in Claim 19 wherein said compartments are less than a microliter in size.

21. Apparatus as defined in Claims 1, 2, 3 or 6 wherein said compartments are formed with a polymeric material which forms means to encaplsulate the liquid of said compartments.

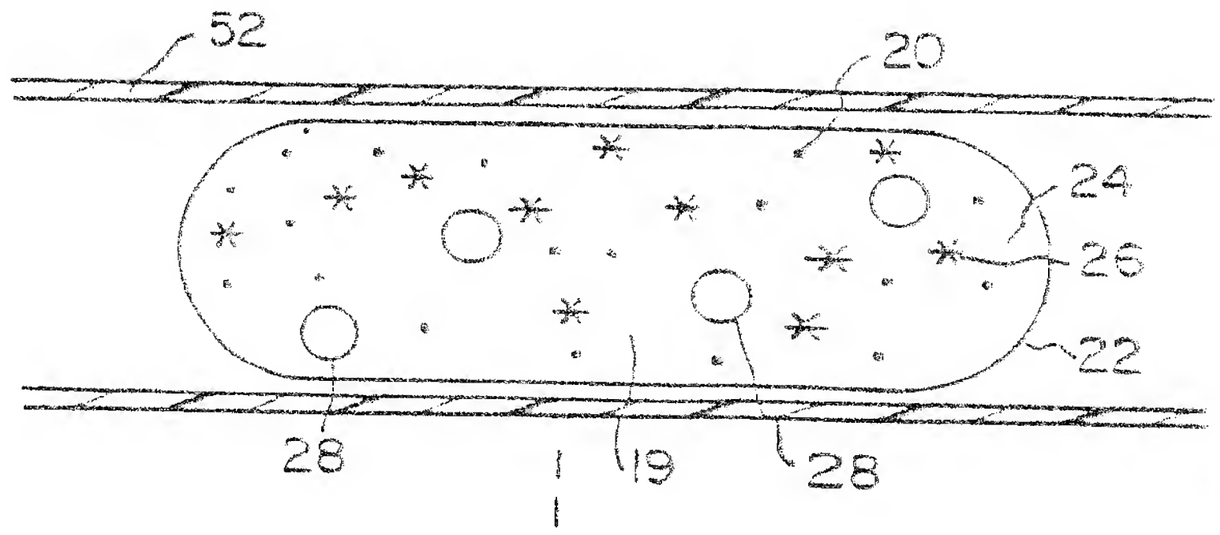

FIG. 1

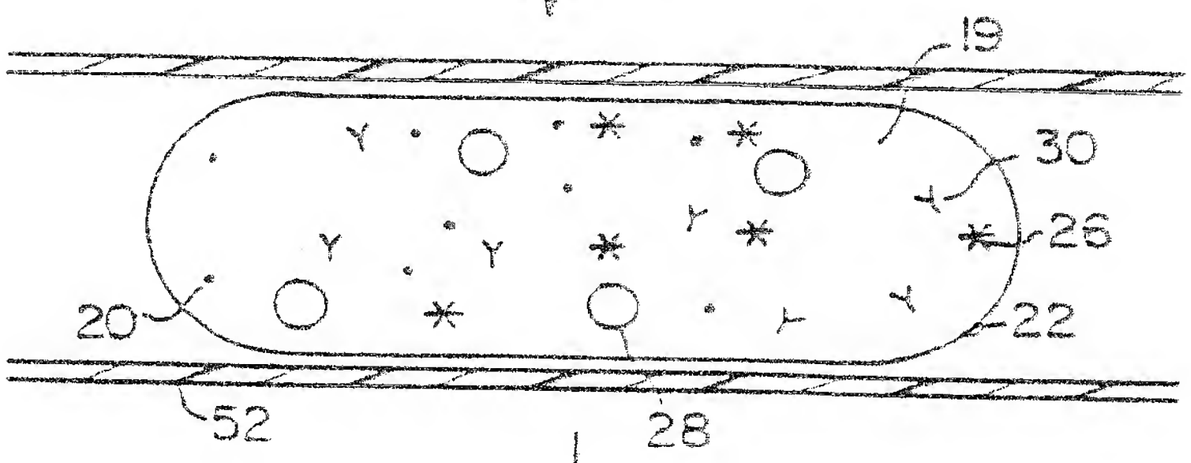

FIG. 2

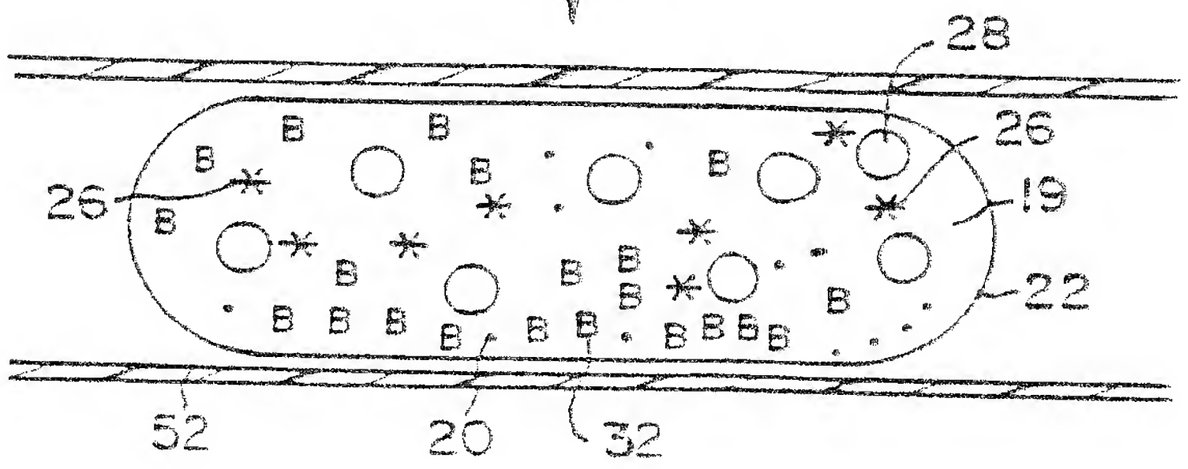

FIG. 3

B: - - Y — *

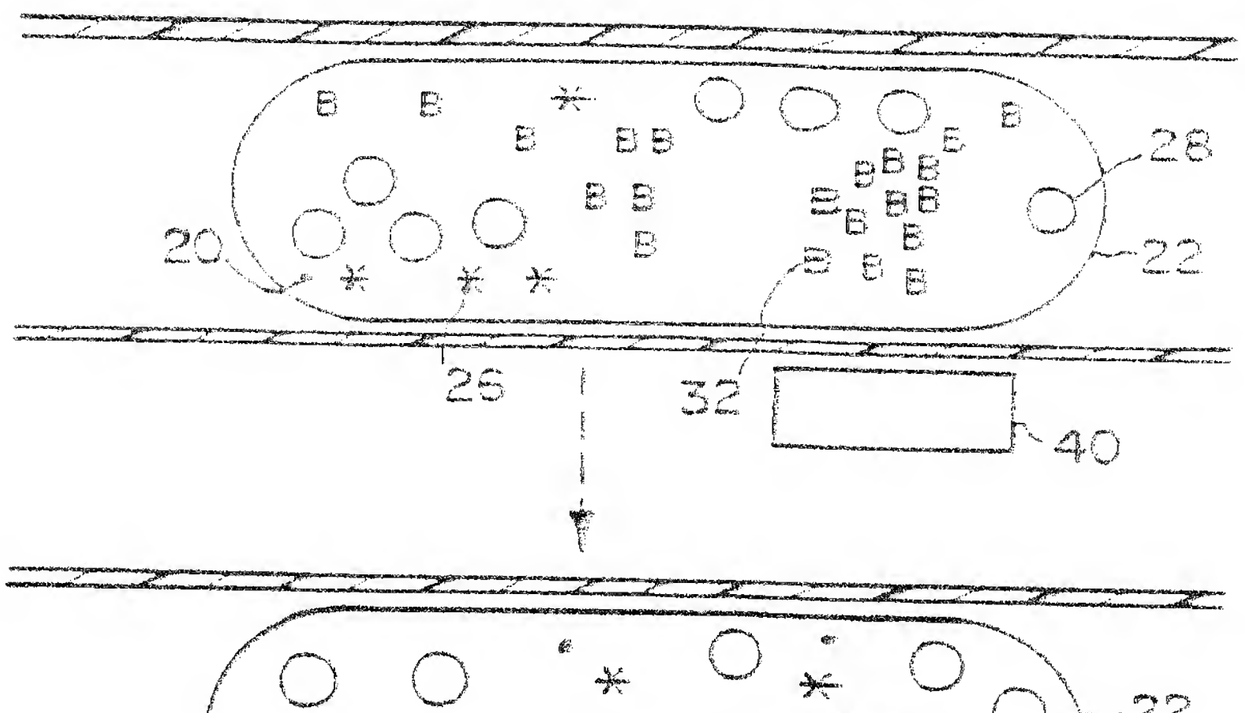

FIG. 4

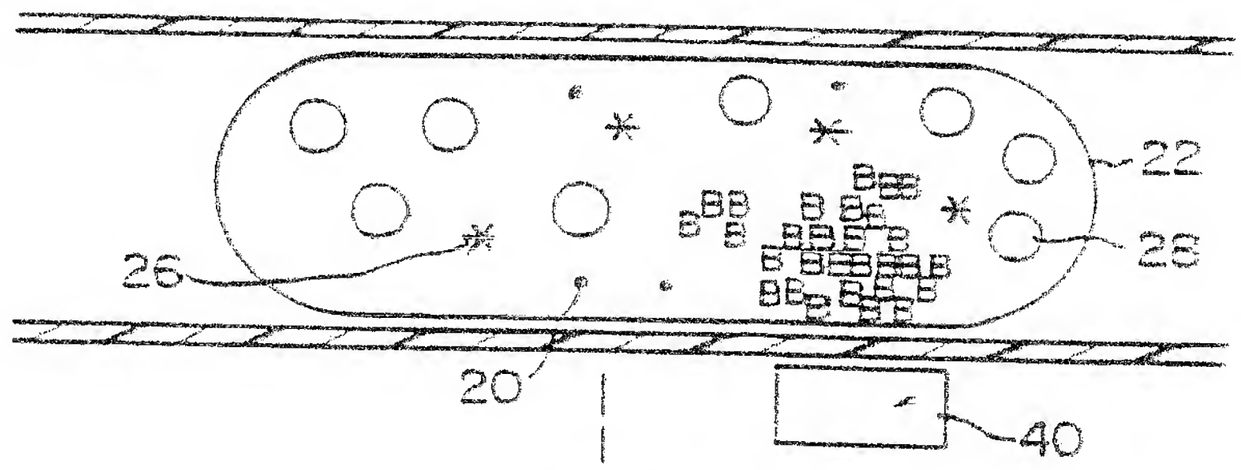

FIG. 5

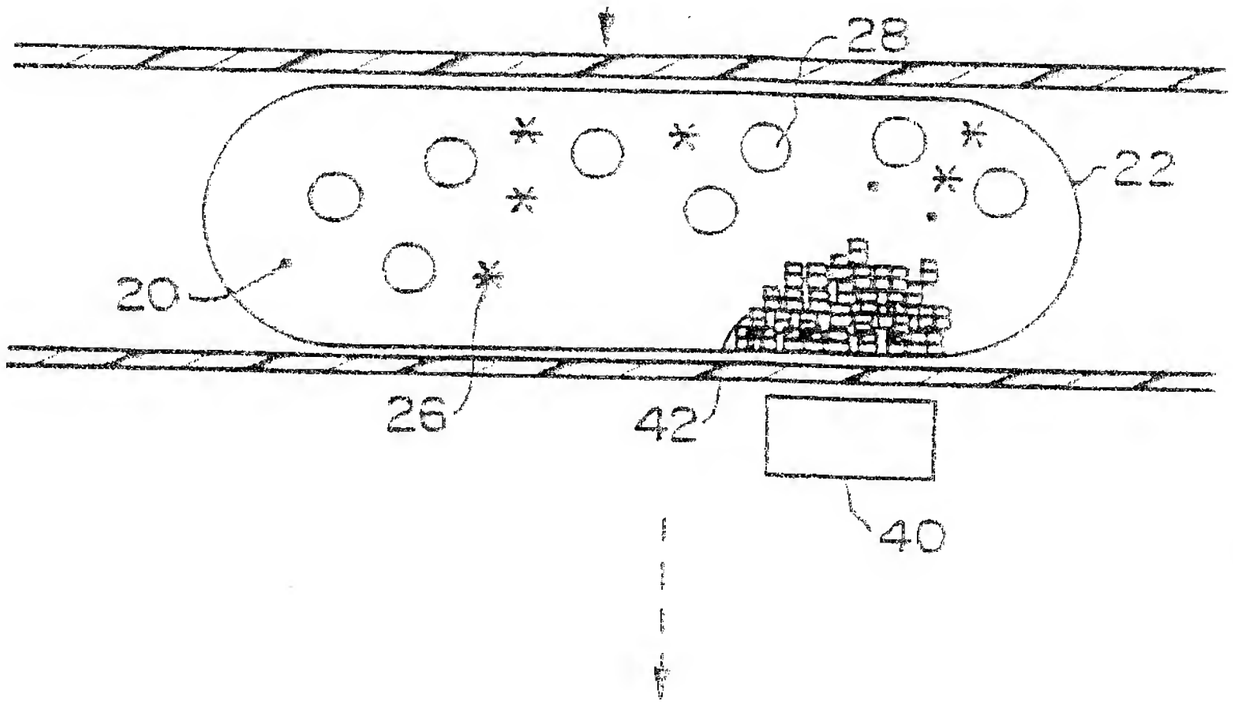

FIG. 6

FIG.7

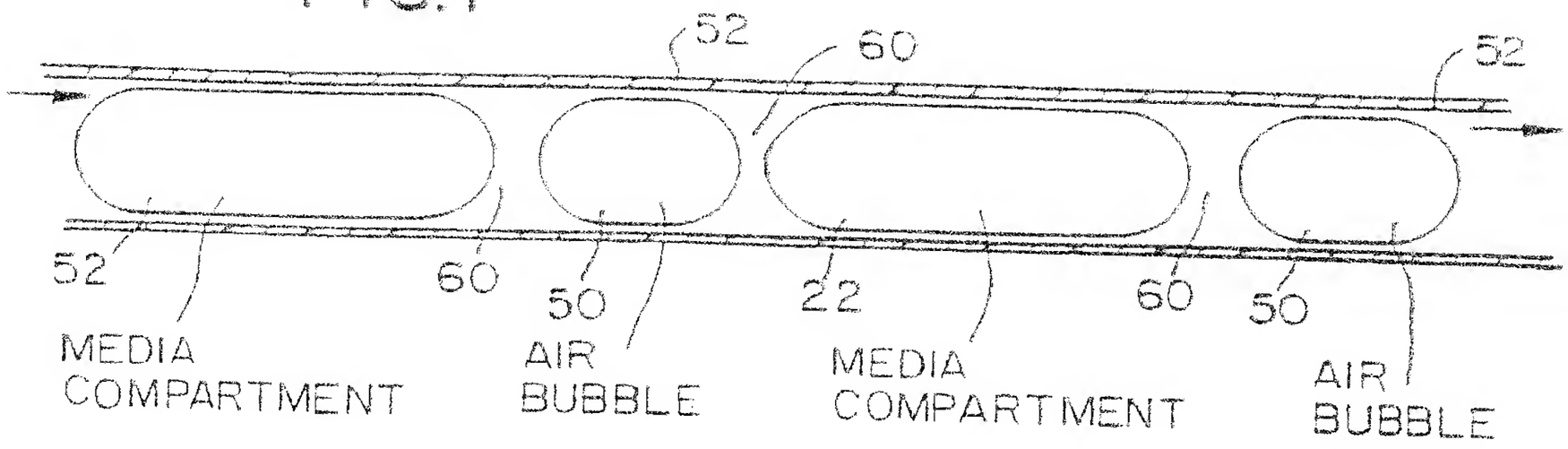

FIG.8

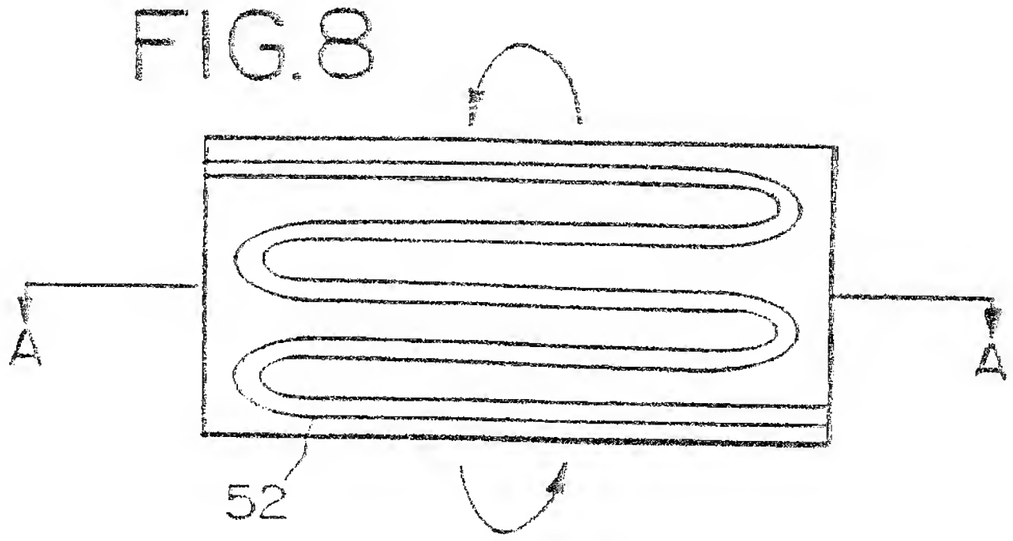

FIG.9